Membrane Dynamics & Bilayer Probes I

431-Pos Board B200
Molecular Dynamics Simulations of Lipid-Linked Oligosaccharide in Membrane Bilayers
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Lipid-linked oligosaccharides (LLO) are intermediates in glycosylation in prokaryotes and eukaryotes. An LLO is composed of a lipid molecule joined by diphosphate to a sugar chain. However, the structure, dynamics, and orientation (with respect to bilayers) of LLO within biological membranes have not been explored previously. Using the recent CHARMM carbohydrate and general force fields, we have constructed two types of LLO: one based on the lipid molecule dolichol (DOL), which is found ubiquitously in eukaryotes, and one based on undecaprenol (UND), which is found in bacteria. We have simulated each LLO type in four different pure bilayer types with different hydrophobic thicknesses and saturations: DLPC (dilauroylphosphatidylcholine), DMPC (dimyristoylphosphatidylcholine), DOPC (dioleoylphosphatidylcholine), and SAPC (stearoylarachidonylcholine). The simulation results of each system will be discussed in terms of density distribution of each component along the membrane normal, RMSD and RMSF of oligosaccharides, oligosaccharides' conformations, DOL and UND conformations, orientations of oligosaccharides with respect to the bilayer normal, and interactions of oligosaccharide with the bilayer.

432-Pos Board B201
Environment Reaction Fields for Lipophilic Fluorophores using Solvatochromic Shifts
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Biological activities such as permeability, and the energetics of protein insertion are governed, in part, by differences in polarity across the phospholipid membrane. Environment polarity can also induce changes in absorption or emission maxima, for a given fluorophore. This is termed solvatochromism. In this study we investigated the relationship between solvatochromic shifts and the membrane polarity for curcumin and aluminum phthalocyanine disulfonic acid ($AlPcS_2$). From the Lippert equation, fluorophore solvatochromism was analyzed using the reaction field of Onsager and later iterations. Each reaction field model predicted the solvatochromic shift based on the solvent dielectric constant, solvent refractive index, and allowed for polarizability of the solute. In addition, the models were extended to consider the effect of dispersion forces. For curcumin, the reaction field of Block and Walker gave the strongest agreement between experimental and predicted values ($r = 0.977$, $p<0.0001$). For $AlPcS_2$, the reaction field of Wertheim, based on statistical mechanics, gave the best agreement ($r = 0.951$, $p = 0.001$), only when dispersion forces and solute polarizability were considered. These results allowed identification of fluorophore environment polarity upon binding to lipid bilayers. In addition, we correlated the model predictions to the Dimroth-Reichardt E_T (30) solvent polarity scale used by Frimer and colleagues. This technique can qualitatively estimate the location of a fluorophore in the lipid membrane. Using the models, curcumin was estimated to be in the acyl chain region of the lipid bilayer, compared to $AlPcS_2$, at the fatty acid carbonyl. This investigation provides a general method to link easily obtained absorption and emission spectra of a given lipophilic fluorophore to its location in a lipid bilayer via classically derived reaction field models.

433-Pos Board B202
Parameterization and Molecular Dynamics Simulations of Nile Red
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The dye 9-diethylamino-5H-benzo[α]phenoxazine-5-one, commonly known as Nile Red, is a fluorescent molecule whose excitation and emission maxima depend on the polarity of the solvent. The dye is mainly used as a probe of the lipid microenvironment. However, the physicochemical properties of a lipid bilayer vary rapidly over the membrane normal. Therefore, atomistic scale information on both the localization and the orientation of the dye molecule in the bilayer is crucial for an accurate molecular level interpretation of fluorescent data.
In this study, we present an all-atom model of the dye that is compatible with OPLS and AMBER force-fields, focusing particularly on the parameterization of the dihedral connecting the diethyl amino group to the phenoxazine ring. We compute the potential of mean force of the dye across a POPC bilayer, and analyze the orientation of the dye molecule along the membrane normal.

434-Pos Board B203
Simulations of the Phase Transition of DPPC Bilayer with and without DPH or TMA-DPH using CHARMM36
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Early CHARMM all-atom force fields showed transition temperatures well above the experimental value of 315K. We simulated 100 ns for DPH and TMA-DPH dyes in DPPC lipid bilayers at atmospheric pressure and temperatures from 300-330K in 5K increments. Shifts were observed between 305K and 315K in area-per-headgroup, bilayer thickness, lipid-tail order parameters, and steady state dye fluorescence anisotropy. Dye effects on these membrane properties were negligible.

435-Pos Board B204
Morphology Transition in Lipid Vesicles: Interaction of In-Plane Order and Topological Defects
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The biological membrane can exhibit complex morphologies driven by compositional heterogeneity, however membrane curvature can also be induced by a defect-driven mechanism. We have found that in the tilted gel phase, complex shapes can form spontaneously even in a membrane containing only a single lipid component as a result of membrane defect formation. To explore this phenomenon we have carried out both experimental observations and coarse-grained computer simulations.
In our study, fluorescence microscopy on giant uni-lamellar vesicles (GUVs) of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) reveals that vesicles can crumple dramatically when cooled from the untilted Lα liquid-crystalline phase into the Lβ' tilted gel phase. Similar behavior is observed in simulation studies of the system.
To explain the phenomenon, we propose that the observed shape evolution is driven by the nucleation of a complex membrane micro-structure that includes topological defects in the tilt orientation. These defects induce non-uniform membrane curvature resulting in a crumpled morphology. Furthermore, we show that competition between curvature change and defect motion can trap vesicles in deeply metastable, defect-rich structures.

436-Pos Board B205
Measuring In-Plane Lipid Phase Dynamics
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We have developed a new time-resolved fluorescence platform which enables us to follow the molecular orientation and dynamics of a lipid monolayer at the air - water interface. This enables us to identify fluorescence probe orientation and dynamic freedom within our membrane model. We have dubbed our technique Dynamic three-Dimensional Fluorescence Microscopy (D3DFM). We demonstrate the novelty and applicability of this device by contrasting the time-resolved fluorescence signal of two different fluorescent probes: NBD-PC and BODIPY bound to a lipid layer (DPPC) and a fatty acid layer (Stearic Acid). We control the phase behavior of our sample by controlling the pressure, and find that unlike the rotational diffusion, the in-plane wobbling is highly sensitive to the the position along the pressure-area isotherm. We believe this is indicative phase coexistence. Other fluorescence techniques are not sensitive to this form of motion due to the geometric constraints of collinear excitation. Using this probe we are able to characterize local dynamic changes that take place upon lipid phase transition, which may be critical for membrane protein recognition and insertion.

437-Pos Board B206
Combined Use of Several Fluorescent Membrane Probes to Study the Subgel Phase and the Effects of Cholesterol Thereon
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Several lines of evidence have indicated that a highly ordered and condensed phase occurs for phosphatidylcholines at low temperatures. Generation of this lamellar subgel phase (Lc) with dipalmitoylphosphatidylcholine (DPPC) depends on the thermal history of the sample; vesicles must be cooled at ~4°C for at least 48 h. Similarly, samples exit the Lc phase slowly at the sub-transition temperature (18°C). Consequently, vestiges of Lc behavior can still be detected at 25°C within 15 min of passing through the transition temperature. We used this hysteresis as an assay for detecting the Lc phase with fluorescent membrane probes by recording the emission spectrum or anisotropy on refrigerated vesicles at 15, 25, 35, and 45°C followed by a return to 35, 25, and finally 15°C. In each case, samples were incubated for 10 min at the new

temperature before data acquisition. The presence of a Lc or Lc-like phase was recognized by lack of reversal of fluorescent properties at 25 or 15°C. The Lc phase was observed for pure DPPC vesicles by merocyanine 540, prodan, and patman but not laurdan or diphenylhexatriene; fluorescence properties of the latter two probes were completely reversible at all temperatures. Inclusion of cholesterol in the vesicles up to 20 mol% caused a linear reduction in the magnitude of the difference between membrane properties detected by these probes before and after vesicle heating. In contrast to previous reports using other techniques, the lack of reversal was not completely eradicated by cholesterol in the range of 20 to 45 mol% suggesting that an Lc-like phase exists for DPPC/cholesterol mixtures. The distinction between probes that can and cannot detect these phases has implications for interpreting the nature of them.

438-Pos Board B207

Octyl-β-D-Glucopyranoside Shows Composition Dependent Disordering Effects in Ternary Lipid Bilayers

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Solubilization of lipid bilayers by the nonionic detergent OG has been well characterized in one and two component lipid systems, but has not been extensively studied in ternary membranes containing mixed fluid phases. In order to understand the effects of membrane order on solubilization by OG, LUVs of pure POPC were compared to those composed of POPC plus varying fractions of sphingomyelin (SM) and cholesterol. Detergent partitioning from the aqueous phase into the bilayer and membrane solubilization were monitored by ITC. The fluorescent membrane probe, DPH, was observed by time-resolved fluorescence intensity and polarization decay in the presence of increasing detergent concentrations to determine the effects on membrane order and dynamics. The results of partitioning experiments showed approximately a 50% decrease in the partition constant between pure POPC and the most ordered samples. ITC solubilization experiments showed clear boundaries for the micelle-bilayer mixed aggregate coexistence region in samples of low initial order, but the distinct thermodynamic signature associated with this coexistence region was not present in the cholesterol and SM rich samples. The average excited state lifetime of DPH, an indicator of water penetration, in vesicles rich in POPC showed a rapid increase at the onset of solubilization, whereas vesicles rich in SM and cholesterol showed a corresponding rapid decrease. Dynamic fluorescence depolarization data analyzed in terms of a Brownian rotational diffusion model revealed an increase in the occupancy of the bilayer midplane by DPH prior to solubilization in less ordered membranes coupled with a constant rotational correlation time. Membranes with higher initial order showed little increase in midplane occupancy and increasing rotational correlation time in the same detergent concentration range, suggesting that two modes of membrane disruption are possible and depend on the lipid makeup of the bilayer.

439-Pos Board B208

Ergosterol and Stigmasterol Interact with Phosphatidylcholine Lipid Bilayers Less Favorably than Cholesterol

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Sterols are a class of membrane lipids that is important to maintain plasma membrane structure and functions in eukaryotic cells. The maximum solubility of sterol in a lipid bilayer is the highest mole fraction of sterol that can be incorporated into a lipid bilayer before sterol precipitates from the bilayer to form crystals. A higher maximum solubility indicates more favorable interactions between the sterol and lipid bilayer. In this study, the maximum solubilities of ergosterol and stigmasterol in DOPC and DSPC lipid bilayers were measured using light scattering and further confirmed using optical microscopy. We found that correlation function of scattering intensities from two independent detectors can be used to sensitively determine the solubility limits of sterols. The validity of our new technique was confirmed by measuring the solubility limit of cholesterol in DOPC and DPPC lipid bilayers. We found that the maximum solubilities of ergosterol and stigmasterol are higher in PC lipid bilayers with saturated chains (DSPC) than that in PC bilayers with unsaturated chains (DOPC). Compared with cholesterol, ergosterol and stigmasterol both have much lower solubility limits in PC lipid bilayers. Our results suggest that minor differences in sterol structure could result in large differences in sterol-PC interactions.

440-Pos Board B209

A New Phase Boundary in Phosphatidylcholine/Cholesterol Bilayers (In the Dimyristoylphosphatidylcholine/Cholesterol Bilayer): EPR and DSC Studies

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A new phase boundary, at cholesterol/dimyristoylphosphatidylcholine (Chol/DMPC) mixing ratio of ~1, was observed by saturation-recovery electron paramagnetic resonance (EPR) spin-labeling method in a multilamellar suspension of DMPC and Chol prepared using a rapid solvent exchange method. With spin-labeled cholesterol analogues (androstane spin label [ASL] and cholestane spin label [CSL]) it was shown that the upper limit of the cholesterol concentration in the liquid-ordered phase of the DMPC membrane is ~50 mol%, above which the excess of cholesterol forms the pure cholesterol bilayer domain (CBD). Thus, the phase boundary at a Chol/DMPC mixing ratio of ~1 separates the region with a single liquid-ordered phase from the region with a coexisting liquid-ordered phase and the CBD. Because the pure cholesterol cannot form the free bilayer in the buffer, the CBD has to be supported by the surrounding phospholipid bilayer saturated with cholesterol. The next phase boundary is defined by the cholesterol solubility threshold (CST) that indicates the amount of cholesterol which can saturate the DMPC bilayer and form the CBD. The excess of cholesterol above the CST forms monohydrate cholesterol crystals which precipitate outside of the lipid bilayer. It was shown by differential scanning calorimetry (DSC) that the CST, which separates the region with a coexisting liquid-ordered phase saturated with cholesterol and the CBD from the region in which cholesterol crystals are formed, is located at the Chol/DMPC mixing ratio of ~2 (~66 mol% cholesterol). The extended phase diagram for the DMPC/cholesterol membrane, covering the region where the membrane is saturated and oversaturated with cholesterol, is proposed.

441-Pos Board B210

The Effect of Electrostatics on the Line Tension at the Edge of a Bilayer

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For bilayers composed of charged lipids placed in an aqueous medium, Betterton and Brenner (Phys Rev. Lett., 2000) showed that the line tension λ at the bilayer edge comprises two contributions: a positive hydrophobic line tension, λ_h, corresponding to the elastic deformation of lipids in the line, and a negative electrostatic line tension, λ_e, corresponding to the capacitive energy of double layer charging. They showed that λ_e depends on a single dimensionless parameter, P, which is the ratio of λ_h and a characteristic scaling of λ_e. For values of P below a critical value of 2, the net line tension becomes negative, implying that fluid bilayers with high surface charge densities should be unable to close up to form vesicles, and that vesicles should be unstable structures. However, we have prepared and imaged stable vesicles with surface charge densities and pH/salt conditions corresponding to $P<<2$; this is inconsistent with the above prediction.

To understand this discrepancy, we revisit the calculations of Betterton and Brenner, with the new, key inclusion of details of the geometry of the bilayer. This results in two additional dimensionless parameters in the problem: α, the ratio of the bilayer thickness to the Debye length, and f, the surface charge density in the edge of the bilayer relative to the planar region of the bilayer. If the surface charge density is uniform over the entire bilayer (f=1) and α is O(1) or higher, we show that λ_h can be positive, i.e. even electrostatics would favor a decrease in the perimeter of an open hole in the bilayer! We delineate conditions under which the net line tension becomes positive, and show that this can happen even when $P<<2$, in agreement with our experimental observations.

442-Pos Board B211

Simulations of the Rupture of Liposomes Near Solid Surfaces

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The behavior of lipid membranes near solid surfaces has a great significance both in medicine and in technology. In spite of the widespread use and study of such membrane phenomena, their theoretical analysis is rather scarce. Our main goal here is to understand the process during which membrane vesicles first adhere to solid surfaces, then rupture (or go through a series of transient ruptures) due to the mechanical tension induced by the adhesion (not only between the membrane and the surface, but also between two adjacent membrane areas), and finally spread along the surface forming a supported lipid bilayer. In our theoretical description we simultaneously consider the dynamics of spontaneous pore opening and closing; volume loss via leakage through the pores; and the advancement of the adhesion fronts. All these processes are supposed to follow an overdamped dynamics and are coupled to each other through membrane tension. Here we identify under which conditions the dynamics leads to the formation of hat shaped geometries with a projecting brim, and compare our results with experimental observations.